GENOMIC AIDED PHARMACOTHERAPY (GAP): A PILOT STUDY IN PATIENTS SUFFERING FROM CHRONIC NON-CANCER PAIN

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NorPM-P1
EFFECT OF RARE GENETIC VARIANTS ON ORGANIC ANION TRANSPORTING POLYPEPTIDE 1B1

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Aims: To examine the effect of single-nucleotide polymorphisms in the transmembrane domains of Organic Anion Transporting Polypeptide 1B1 (OATP1B1, SLCO1B1) on its activity.
Methods: 8 naturally occurring variants in the transmembrane domains of OATP1B1 and a common variant (c.521T>C) which served as a control were selected. The mutations were inserted in the wildtype OATP1B1 gene by site-directed mutagenesis. The Bac-to-Bac baculovirus system was used to transiently express the variant proteins in mammalian cells (HEK293). Dichlorofluorescein and estrone-3-sulphate were used as substrates in cellular uptake studies to test the activity of the variants. The effects of the polymorphisms on expression levels and localization of the variants were studied with Western blotting and immunofluorescence microscopy.
Results: The transport activity of some of the variants in the transmembrane region was significantly impaired compared to wildtype OATP1B1, while others exhibited normal or even enhanced uptake of the study substrates. Immunofluorescence studies in cells indicate impaired localization for some of the variants, which could at least partly explain the decrease in activity.
Conclusion: The activity of OATP1B1 may be altered due to rare single nucleotide polymorphisms in the transmembrane domains.

NorPM-P2
CLOPIDOGREL MARKEDLY INCREASES DASABUVIR EXPOSURE, AND RITONAVIR INHIBITS THE BIOACTIVATION OF CLOPIDOGREL

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Aims: The purpose of this clinical study was to characterize the effect of the CYP2C8 inhibitor clopidogrel on the pharmacokinetics of the CYP2C8 substrate dasabuvir. Because dasabuvir is routinely used in an antiviral drug regimen containing ritonavir, a strong CYP3A4 inhibitor, the secondary aims were to explore how ritonavir modifies clopidogrel-dasabuvir interaction and whether ritonavir affects the bioactivation of clopidogrel, which is partly CYP3A4-mediated.
Methods: In a four-phase cross-over study, twelve healthy volunteers were administered clinical doses of clopidogrel, ritonavir, their combination, or placebo as pretreatments, and a single dose of dasabuvir. The plasma concentrations of dasabuvir, clopidogrel, their metabolites, and ritonavir were determined with liquid chromatography/mass spectrometry. The antiplatelet effect of clopidogrel was measured with a turbidimetric optical detection system.
Results: Clopidogrel increased the geometric mean $AUC_{0\text{-}\infty}$ of dasabuvir 4.7-fold (90% CI of geometric mean ratio 3.2–6.7-fold; $p = 8 \times 10^{-7}$), compared with placebo. When clopidogrel and ritonavir were co-administered, dasabuvir $AUC_{0\text{-}\infty}$ was 3.9-fold (90% CI 2.8–5.5-fold; $p = 2 \times 10^{-6}$) of that during the ritonavir phase. Ritonavir decreased the $AUC_{0\text{–}4\ h}$ of clopidogrel active metabolite by 51% (90% CI 39–61%; $p = 0.0001$), and average platelet inhibition diminished from 51% to 31% by ritonavir (mean difference 90% CI −27% to −12%; $p = 0.0007$).
Conclusion: Clinical doses of clopidogrel markedly augment dasabuvir exposure and inhibit the CYP2C8-mediated formation of the dasabuvir metabolite, M1. Therefore, the risk for dasabuvir adverse effects, i.e. QTc prolongation, may increase if clopidogrel is used concomitantly with dasabuvir, irrespective of ritonavir. In addition, dasabuvir can serve as a CYP2C8 probe substrate for drug interaction studies. Lastly, ritonavir can reduce the antithrombotic efficacy of clopidogrel. Thus, concurrent use of clopidogrel and dasabuvir is best avoided.

NorPM-P3
UNDERSTANDING DRUG REACTIONS USING GENOME SEQUENCING (UDRUGS) STUDY: A NEW ZEALAND INITIATIVE TO INVESTIGATE THE GENETICS OF ADRS

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Aims: UDRUGS is a New Zealand initiative to systematically collect samples from people who have had adverse drug reactions (ADRs) to characterize known or identify novel genetic variants associated with rare, unusual, and severe ADRs.
Methods: Participants for the UDRUGS study are largely recruited via referral through clinicians working in Christchurch hospital, but also from clinicians across New Zealand. Written consent is sought from all patients referred to the UDRUGS study. Genetic analysis largely involves Sanger sequencing of candidate genes. We have also used whole exome sequencing (WES) or whole genome sequencing (WGS) for a subset of cases. We communicate relevant research findings back to the participants and/or the referring clinician citing guidelines from the Clinical Pharmacogenetics Implementation Consortium (CPIC).
Results: To date we have collected ~100 samples across 12 general drug classes. The majority of cases recruited have been for ADRs to antidepressants, drugs affecting immune response (azathioprine) and cardiovascular medication (statins and medication used for hypertension). For antidepressants, we have genotyped 37 patients, and observed that 65% of the cohort had at least one non-functional *CYP2D6* allele ($p < 0.01$, compared with *CYP2D6* EXAC NFE). In addition we have carried out WES on participants with recurrent statin-myalgia, and participants with ACEi induced angioedema. For both cohorts our analyses have identified rare variants which require further validation.
Conclusion: We have developed a programme for studying severe, rare or unusual ADR cases resulting from pharmacological treatment. Genomic analyses should eventually identify most genetic variants that predispose to ADRs, enabling *a priori* detection of such variants with high throughput DNA tests. Establishing UDRUGS, a New Zealand DNA bank that can receive ADR samples and associated clinical data, will ensure we contribute to and benefit from such research.

NorPM-P4
DIFFERENCES IN SENSITIVITY TO DETECT TIME-DEPENDENT INACTIVATION OF CYP2C8 BETWEEN RECOMBINANT EXPRESSED ENZYMES AND HUMAN LIVER MICROSOMES

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Aims: Clopidogrel acyl-β-D-glucuronide causes strong time-dependent inactivation of CYP2C8 in human liver microsomes (HLMs). However, in preliminary studies on the inactivation mechanism, we were unable to detect inactivation of CYP2C8 by clopidogrel glucuronide in Supersomes. Therefore, we investigated the performance of different CYP2C8 sources in detection of time-dependent inactivation.
Methods: Time-dependent inactivation of CYP2C8 by clopidogrel acyl-β-D-glucuronide (100 μM), gemfibrozil 1-O-β-glucuronide (100 μM) and amiodarone (100 μM) was studied in HLMs and three recombinant human CYP2C8 preparations (Supersomes, Bactosomes and EasyCYP Bactosomes) using amodiaquine N-demethylation as the marker reaction. The inactivation kinetics of CYP2C8 by clopidogrel acyl-β-D-glucuronide (5–250 μM) was studied in Supersomes and Bactosomes.
Results: Time-dependent inactivation of CYP2C8 by amiodarone and gemfibrozil glucuronide was observed in all enzyme preparations, with moderate variability between preparations. However, the extent of inactivation by clopidogrel glucuronide varied markedly between different preparations, as well as between different Supersome lots. While clopidogrel glucuronide (100 μM) caused strong inactivation of CYP2C8 in HLMs (72% and 16% inhibition with and without 30-min preincubation, respectively) and in one Supersome lot (54% and 0% inhibition with and without preincubation, respectively), significant inactivation could not be reliably detected in two other lots. In Bactosomes, the K_I and k_{inact} of clopidogrel glucuronide (14 μM and 0.054 min^{-1}) were close to those determined previously in HLMs (9.9 μM and 0.047 min^{-1}).
Conclusion: The results show that there are differences between enzyme sources and between lots of recombinant enzymes in sensitivity to detect inactivation of CYP2C8. The risk of false negatives should be taken into account when recombinant CYP enzymes are used to identify time-dependent inactivators of CYP enzymes.

NorPM-P5
NEW CONCEPTS IN DISEASE CLASSIFICATION, TOWARD GENE BASED DIAGNOSIS, SUBTYPES AND PREDICTION OF DRUG RESISTANCE IN MALIGNANT B-CELL DISEASES

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Aims: We perceive the future of cancer treatment as being in the development of "personalised medicine" strategies and malignant B-cell diseases (MBCD) is an excellent example, where this approach can be clinically validated.
Our concept is to take individual gene expression variability and new classifications into account by new strategies of disease diagnosis, new subtyping and new drug specific prediction. Here we present a summary of our work with the primary aims to give useful information to clinical decision making.
Methods: Tumor biopsies from 4,226 patients with Affymetrix U133 plus 2.0 microarray mRNA expression datasets, in collaborations and/or on line from 21 newly diagnosed/untreated cohorts with lymphoid leukemia, non-Hodgkin lymphoma or multiple myeloma. Powerful methods for phenotyping by multi omics, diverse preclinical cell line models and bioinformatic tools for large data set analysis allowed us to address new classifications exemplified by diagnostic analysis gene signature (DAGS), B-cell associated gene signature (BAGS) and resistant gene signature (REGS) assignment of individual tumors.
Results: The resultant tumor assignments in the available clinical datasets exhibited the following:
1. Individual DAGS have a significant *diagnostic accuracy*; (Table 1).
2. Individual BAGS *disease subtypes* have prognostic impact, and therefore reflect pathogenesis; (Figure 1).
3. Individual REGS for specific *drug resistance* has predictive information documented by its prognostic impact; (Figure 2).

Consequently, we have prepared an R-package based tool: http://www.hemaclass.org/ relevant for implementation of PM in the clinical setting.
Conclusion: The results "proves our concept" that individual variability and new classification by gene expression may allow more precise diagnostics, subtyping and identify drug specific resistance that will reduce overtreatment, increase the "Value in Health Care" and validate the PM strategy and goals.

NorPM-P6
HEMATOLOGICAL CANCER PATIENTS' POSITION ON PRECISION MEDICINE – A QUESTIONNAIRE SURVEY

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Aims: Precision Medicine has gained increased medical and political attention in recent years. In Denmark, this has resulted in The National Strategy for Precision Medicine 2017–2020 and development of a national genome center. In 2016, the Danish Ministry of Health and Danish Regions performed a population-based survey of the public's position on precision medicine. However, to our knowledge no study has investigated patients' position on this area. Therefore, the aim of this study was to investigate hematological cancer patients' position on precision medicine compared to the healthy population.
Methods: Data was collected using a questionnaire developed by ADVICE Communication Agency for the population-based survey and on behalf of the Danish Ministry of Health and Danish Regions. Eligible participants were hematological cancer patients affiliated to the Department of Hematology, Aalborg University Hospital. The questionnaire was administered electronically through REDCap or on paper. Data was collected anonymously.
Results: Between October 2017 and January 2018, we enrolled 286 patients (median age; men 66 years, women 67 years). The study population had a higher median age compared to the public (n = 1005) (median age; men 60.5 years, women 53 years) and a lower education level. We found that 84% of the patients had none or limited knowledge about gene testing compared to 55% of the public (p = 0.0001). 97% of the patients found gene testing and precision medicine interesting compared to 52% of the public (p = 0.0001). Furthermore, 92% of the patients were positive towards genetic research compared to 40% of the public (p = 0.0001).
Conclusion: We have identified a difference in the knowledge level between hematological cancer patients and the public. A difference, which should be addressed in order to optimize and support patient dialogue in a precision medicine context.

NorPM-P7
MEDICATIONS DURING PREGNANCY: EFFECTS ON NEURODEVELOPMENTAL DISORDERS IN CHILDHOOD AND EPIGENETIC OUTCOMES

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Aims: To present the PharmaTox initiative and our on-going studies to assess effects of pharmaceuticals on human neurodevelopment and neurotoxicity
Rational: The fetal period is believed to be one of the most sensitive periods for brain development. During this period, neurons are created, differentiate and migrate to form the various parts of the brain. The theoretical potential of neurotoxicity of pharmaceuticals is related to their ability to pass the blood-brain barrier and impact on the developing fetal nervous system. The PharmaTox Strategic Research Initiative was established in 2015 uniting strong research groups in pharmacoepigemiology, toxicology, epigenetics, bioinformatics and statistics at the University of Oslo, Norway.
Methods: A primary data source is epidemiological data from the Norwegian Mother and Child (MoBa) cohort, along with corresponding genetic and epigenetic data derived from blood samples of infants in MoBa. Moreover, to give biological plausible molecular and cellular mechanisms of neurotoxicity, a CNS pharmacology safety model (chicken embryo) and human embryonic stem cells are used. Advanced statistical methods, medical bioinformatics and microarray technology are an integrated part of this research.
Results: Several results have been published in relation to paracetamol use in pregnancy. An association between long-term use of paracetamol with increased risk of ADHD was recently published, as well as findings of altered DNA-methylation patterns among children with ADHD diagnosis prenatally exposed to long-term use of paracetamol. Preliminary results from the chicken embryo model suggests that paracetamol and other medications change the expression of specific microRNAs.
Conclusion: Novel insight into neurotoxicity of pharmaceuticals can be obtained by using a translational approach, advanced statistical methods, medical bioinformatics and microarray technology on epidemiological and human genetic data in combination with experimental in vivo and intro models.

NorPM-P8
AN OPTIMIZED PREDICTION FRAMEWORK TO ASSESS THE FUNCTIONAL IMPACT OF PHARMACOGENETIC VARIANTS

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Aims: Prediction of phenotypic consequences of mutations constitutes an important aspect of precision medicine. Current computational tools mostly rely on evolutionary conservation and have been calibrated on variants associated with disease, which poses problems for assessment of variants in poorly conserved pharmacogenes. Therefore, we aimed to develop a functional prediction framework optimized for pharmacogenetics assessments that significantly outperformed current predictive algorisms.
Methods: Experimental functionality data of 244 pharmacogenetic missense variants distributed across 21 ADME genes were obtained to optimize 18 current functionality prediction models. Specifically, 123 training variants were randomly assigned for the optimization and the best combination of optimized models were validated by the rest 121 variants.
Results: Compared to current models with low predictive power, our prediction model achieved 92% sensitivity and 95% specificity for loss-of-function and functionally neutral variants. This result was also confirmed in an independent validation cohort.
Conclusion: This ADME-optimized prediction framework significantly improves *in silico* functionality assessment of pharmacogenetic variants, thereby facilitating the translation of uncharacterized variants into pharmacogenetic recommendations and providing a further step towards the leveraging of Next-Generation Sequencing data for the personalization of pharmacological treatment.

NorPM-P9
EFFECTS OF GENETIC VARIANTS OF HUMAN NICOTINIC RECEPTOR SUBUNITS CHRNA3, CHRNA4, CHRNA5 ON NICOTINE DEPENDENCE AND SMOKING CESSATION

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Aims: To investigate the effects of genetic variants in nicotinic receptor subunits of *CHRNA3* rs578776, *CHRNA4* rs1044396-rs1044397, and *CHRNA5* rs16969968 on the heaviness of nicotine dependence and outcomes of smoking cessation treatments at the 12th week in a Turkish population.
Methods: We recruited 130 smokers and 130 non-smokers for this study. The heaviness of nicotine dependence was determined by Fagerström Test for Nicotine Dependence and CO levels. Scores e7 were considered as high nicotine dependence (n = 60), scores <7 were considered as medium or low nicotine dependence (n = 70). Treatment groups were nicotine replacement therapy (NRT, n = 40), bupropion (n = 47), bupropion+NRT (n = 15) and varenicline (n = 28). PCR-RFLP was used for genotyping.
Results: We found an association between the heaviness of nicotine dependence and AA genotype for the *CHRNA4* rs1044396 variant (p = 0.02) and, with A allele for the *CHRNA5* rs16969968 variant (p = 0.003). Frequencies for the *CHRNA4* rs1044396 GG, GA, and AA genotypes were 30%, 53.3%, 16.7% in high nicotine-dependent smokers; 22.9%, 52.9%, 24.3% in medium or low nicotine-dependent smokers; 30%, 34.6%, 35.4% in non-smokers, respectively. Frequencies for the *CHRNA5* rs16969968 G and A alleles were 58.3%, 41.7% in high nicotine-dependent smokers; 42.1%, 57.9% in medium or low nicotine-dependent smokers; 40%, 60% in non-smokers, respectively. We also found an association between T allele for the *CHRNA3* rs578776 genetic variant and failure of bupropion+NRT treatment (p = 0.033). C and T allele frequencies in the bupropion+NRT group were: 83.3% and, 16.7% in quitters; 44.4% and, 55.6% in non-quitters, respectively.
Conclusion: AA genotype for the *CHRNA4* rs1044396 variant and A allele for the *CHRNA5* rs16969968 variant was associated with a lower level of nicotine dependence. The T allele for the *CHRNA3* rs578776 genetic variant was associated with failure of smoking cessation therapy in patients treated with bupropion+NRT. Supported by Hacettepe University grant TSA-2017-12810.

NorPM-P10
EFFECTS OF A GENETIC POLYMORPHISM OF HUMAN CYP2B6 ON NICOTINE DEPENDENCE AND SMOKING CESSATION THERAPY

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Aims: CYP2B6 is responsible for the metabolism of nicotine and bupropion. The aim of this study was to investigate the effects of *CYP2B6* rs2279343 genetic polymorphism on the heaviness of nicotine dependence and smoking cessation after 12 weeks of treatment in a Turkish population.
Methods: A total of 260 volunteers; 130 smokers and 130 non-smokers were recruited. Determination of the heaviness of nicotine dependence in the smokers was performed by Fagerström Test for Nicotine Dependence and verified by breath CO measurements. Scores ɐ7 were considered as high nicotine dependence (n = 60), scores <7 were considered as medium or low nicotine dependence (n = 70). There were four treatment groups: nicotine replacement therapy (NRT, n = 40), bupropion (n = 47), bupropion+NRT (n = 15) and varenicline (n = 28). PCR-RFLP was used for genotyping.
Results: Frequencies for the AA, AG and GG genotypes were similar in all groups: 43.3%, 48.3%, 8.3% in high nicotine-dependent smokers; 45.7%, 44.3%, %10 in medium or low nicotine-dependent smokers; 46.2%, 40.8%, 13.1% in non-smokers, respectively ($p \geq 0.05$). Also, we found no association between *CYP2B6* rs2279343 polymorphism and cessation rates of smokers after 12 weeks of treatment ($p \geq 0.05$).
Conclusion: This study showed no association of *CYP2B6* rs2279343 polymorphism with the heaviness of nicotine dependence and with smoking cessation after 12 weeks of treatment.
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NorPM-P11
PROTEOMIC ANALYSIS OF SERUM FOR EARLY BIOMARKERS OF HEPATOLENTICULAR DEGENERATION IN MALE ADOLESCENTS

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Aims: The rare autosomal recessive defects in the copper transporting ATPase gene (ATP7B), known as Wilson's disease (WD), leading to increased accumulation of copper in liver, cornea and basal ganglia and cause liver cirrhosis combined with variable neuropsychological manifestations, but early and personalized diagnosis to differentiate between asymptomatic and progressive stage of WD (hepatolenticular degeneration) in humans are still challenging issue.
Methods: Proteome profiles of venus blood serum from WD male adolescent subjects (mean age 13.2 ± 1.2 years) were separated by 2-D gel electrophoresis and identified by peptide mass fingerprinting (MALDI-MS). Western blotting and immunohistochemistry were used to validate the protein expression *in situ*. Dot-blot was used to prove the high-level expression of fibrinogen in both homozygous and heterozygous WD subjects and compared to age matched healthy controls (HC) and patients with confirmed blood stasis cardiovascular symptoms (BSC). The partial N-terminal amino acid residues sequencing and protein database searching was used to verify the 2-DE results.
Results: Total 31 protein spots with difference between WD and HC subjects were counted and identified. Specifically, 22 protein spots covering fibrinogen-γ family, fibrinogen-α group, fibrinogen-β chain, Apo-family protein (A1, E, J), acute phase proteins (α2-macroglobulin, haptoglobin-α), antithrombin III, complement C3 fragment, serotransferrin, transthyretin and orosomucoid-2 were found in the significantly more abundant quantity in the WD subjects.
Conclusion: The initial liver cirrhosis accompanied with acute oxidative stress caused by continuos development of WD may account for the abnormal increase of fibrinogen level (significantly more than observed in BSC) which could lead to the subtle but clinically relevant disturbances in the function of coagulation system of WD patients, thus our data support some results reported by Schaefer et al. (2015).

NorPM-P12
INCREASED SUM CONCENTRATION OF VENLAFAXINE AND O-DESMETHYLVENLAFAXINE AND RISK OF SIDE EFFECTS IN COMBINED CYP2D6/CYP2C19 POOR METABOLIZERS

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Aims: The aim of this study was to evaluate the combined impact of CYP2D6 and CYP2C19 genotypes on the active sum of venlafaxine and O-desmethylvenlafaxine serum concentrations.
Methods: Serum concentrations of venlafaxine and O-desmethylvenlafaxine measured in patients with available *CYP2D6 and CYP2C19* and genotypes were included from a Therapeutic Drug Monitoring (TDM) service at Diakonhjemmet Hospital in Oslo (Norway). Patients were divided into different subgroups according to genotypes: Extensive metabolizers (EM), intermediate metabolizers (IM) and poor metabolizers (PM) of CYP2D6 and CYP2C19, respectively. The impact of CYP2D6 and CYP2C19 genotypes on dose-adjusted sum concentration of venlafaxine and O-desmethylvenlafaxine, the pharmacological active moiety, was evaluated using multiple linear regression analysis.
Results: Overall, 980 patients were included in this study. The dose-adjusted sum concentration of venlafaxine and O-desmethylvenlafaxine was significantly associated with CYP2D6 and CYP2C19 genotypes ($p < 0.001$). The number of combined carriers of either CYP2D6 and CYP2C19 IMs or PMs was in total 62 (6.3% of the total population). For combined CYP2D6/CYP2C19 IMs (n = 58; 5.9% of total) and PMs (n = 4; 0.4% of total), the sum concentration increased by 40% and 380%, respectively, compared to combined EMs (n = 256) ($p < 0.001$).
Conclusion: Combined CYP2D6 and CYP2C19 PMs had a 5-fold higher sum concentration of venlafaxine and O-desmethylvenlafaxine compared to combined EMs. The subpopulation of combined PMs, representing 1 per 200 Caucasian patients, is therefore at substantial risk of side effects during venlafaxine treatment.

NorPM-P13
DOSE DEPENDENT CYP2D6 INHIBITION BY BUPROPION AND LEVOMEPROMAZINE

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Aims: The aim of this study was to compare the dose-dependent CYP2D6-inhibitory effect of bupropion (BUP) and levomepromazine (LEV) using O/N-desmethylvenlafaxine as biomarker.
Methods: Serum concentrations of O-desmethylvenlafaxine and N-desmethylvenlafaxine measured in patients co-treated with different CYP2D6-inhibitors were retrieved from a therapeutic drug monitoring database at Diakonhjemmet Hospital (Oslo, Norway). Metabolic ratio

(MR) O-desmethylvenlafaxine/N-desmethylvenlafaxine in two dose groups of BUP (≤150 mg and >150 mg) and LEV (≤50 mg and >50 mg) were compared with a reference group of patients receiving the potent CYP2D6 inhibitors fluoxetine (FLU) or paroxetine (PAR). Groups were compared using a multiple linear regression model.
Results: A total of 158 patients were included in this study. All patients were treated with venlafaxine in combination with one of the following CYP2D6 inhibitors: BUP ≤150 mg/day (n = 54), BUP>150 mg/day (n = 35), LEV ≤50 mg/day (n = 20), LEV>50 mg/day (n = 32) or FLU/PAR (n = 17). MR in patients receiving high doses of BUP or LEV were not significantly different from the reference group ($p > 0.15$), while patients receiving low doses BUP or LEV had higher MR than the reference groups (+417% $p < 0.001$ and +297% $p < 0.001$ respectively).
Conclusion: Doses in the higher therapeutic range of bupropion and levomepromazine show a similar CYP2D6 inhibition as the potent inhibitors fluoxetine and paroxetine. The dose-dependent CYP2D6-inhibitory effect of bupropion and levomepromazine is important to consider regarding interaction risk in clinical practice.

NorPM-P14
ENANTIOSPECIFIC PHARMACOGENOMICS OF FLUVASTATIN

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Aims: Large interindividual variability exists in the pharmacokinetics and effects of fluvastatin. Our aim was to investigate how sequence variations in pharmacokinetic genes affect fluvastatin exposure.
Methods: A single 40 mg oral dose of racemic fluvastatin was administered to 200 healthy volunteers. Plasma 3R,5S- and 3S,5R-fluvastatin enantiomer concentrations were measured using liquid chromatography-tandem mass spectrometry and 379 genes related to pharmacokinetics were fully sequenced using massive parallel sequencing.
Results: The *CYP2C9* decreased-function allele *CYP2C9*3* (rs1057910; c.1075A>C; p.Ile359Leu) was associated with increased area under the plasma concentration-time curve (AUC) of both fluvastatin enantiomers. The AUC of 3R,5S-fluvastatin was 67% ($p = 3.44 \times 10^{-9}$) and that of 3S,5R-fluvastatin was 94% ($p = 2.80 \times 10^{-12}$) larger per copy of the variant allele. In addition, the *SLCO1B1* decreased-function rs4149056 (c.521T>C; p.Val174Ala) variant increased the AUC of 3R,5S-fluvastatin by 34% per minor allele ($p = 7.55 \times 10^{-8}$), but had no effect on 3S,5R-fluvastatin. *In vitro*, however, the two enantiomers were similarly transported by OATP1B1 (encoded by *SLCO1B1*).
Conclusion: These results indicate that *CYP2C9*3* plays an important role in determining interindividual variability in fluvastatin pharmacokinetics. Furthermore, *SLCO1B1* genotype affects the pharmacokinetics of the pharmacologically more active 3R,5S-enantiomer of fluvastatin. This knowledge may aid in individualizing cholesterol-lowering therapy.

NorPM-P16
MECHANISM OF IMATINIB-RESISTANCE – MICRORNA-212/ ABCG2-AXIS CONTRIBUTES TO DEVELOPMENT OF IMATINIB-RESISTANCE IN LEUKEMIC CELLS

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Aims: The hematopoietic malignancy chronic myeloid leukemia (CML) is caused by translocation between chromosomes 9 and 22 leading to formation of the Philadelphia chromosome and the *BCR-ABL* fusion gene. Although the tyrosine-kinase inhibitor (TKI) imatinib led to tremendous treatment success, the development of resistances against imatinib is an emerging problem. Beside BCR-ABL-dependent mechanisms (e.g. BCR-ABL amplification/overexpression, point mutations), BCR-ABL-independent mechanisms occur, which can be linked in part to dysregulation of ATP-binding cassette (ABC)-transporters leading to increased TKI efflux, potentially caused by changes in microRNA expression or DNA-methylation. In an *in vitro*-imatinib-resistance model using K-562 cells, microRNA-212 was identified to be deregulated and inversely correlated to ABC-transporter ABCG2 expression, targeting its 3'-UTR.
Methods: To analyze the functional impact on drug sensitivity, transfection experiments with microRNA-mimics and –inhibitors were performed to investigate their effect on imatinib-susceptibility in sensitive and resistant leukemic cell lines. In addition the methylation status of *miR-212* and *ABCG2* promoter regions were analyzed.
Results: Under imatinib treatment, miR-212 inhibition led to enhanced cell viability ($p = 0.01$), reduced apoptosis ($p = 0.01$) and cytotoxicity ($p = 0.03$). These effects were only observed in treatment-naïve cells but not in cells resistant to various imatinib-concentrations (0.1 μM to 2 μM). In treatment-naïve cells inhibition of miR-212 resulted in ABCG2 upregulation and increased ABCG2-dependent efflux. In addition, in 0.5 and 2 μM IM-resistant sublines we observed a hypermethylation of the *miR-212* promoter region, whereas the *ABCG2* gene methylation status was not altered.
Conclusion: In summary, the miR-212/ABCG2-axis influences imatinib-susceptibility and contributes to development of imatinib-resistance. Our data reveal new insights into mechanisms initiating imatinib-resistance in leukemic cells.

NorPM-P18
IMMUNOGENETICS AND PROGNOSIS OF PROSTATE CANCER: FIRST RESULTS

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Aims: Many efforts are still necessary to determine robust pharmacogenetics (PGx) biomarkers to be implemented in the clinical practice to advance the field of personalized medicine in cancer treatment. In this regard, one clinical model of interest is prostate cancer (PCa). The primary aim of this study is the identification of new biomarkers of biochemical recurrence (BCR) analyzing polymorphisms (SNPs) of genes involved in immune system. The secondary aim is the determination of biomarkers of overall survival (OS). These new biomarkers, if validated, could be implemented in the electronic clinical record to personalize patients' treatment.
Methods: 418 Caucasian patients affected by clinically localized PCa treated with RT as primary therapy were enrolled. The genetic analyses of 447 SNPs in genes of immune system were determined in germline DNA samples using a VeraCode (Illumina) platform. Multivariate COX regression was applied to identify the prognostic role of SNPs in terms of both BCR and OS. Significant associations were determined according to p-value (<0.05) and q-value (<0.15), defined according to the False discovery rate (FDR) method.
Results: 19 SNPs were significantly associated with BCR. Interestingly, these SNPs addressed in an independent way the key role of *PDL1*, *VEGFR*, *FOXO3*, *SMAD3*, *SMAD2*, and *IL4R*, that are involved in angiogenesis and TGFβ pathway.

The rs3918262-*MMP9*, rs7692791-*VEGFR*, and rs2034967-*VEGFR* can predict OS, reinforcing the importance of angiogenesis in this clinical setting.
Conclusion: This study identified the key role of SNPs impacting immune system activity in the prediction of BCR and OS, highlighting the relevance of angiogenesis in this setting. The prognostic role of these SNPs is going to be tested in a replication set of 131 PCa patients. These analyses are needed to hopefully offer new biomarkers to optimize PCa patients' management through the development of innovative and user-friendly IT tools.

NorPM-P19
DEVELOPMENT OF AN INTEGRATED HEALTH IT PLATFORM FOR A PRECISE AND COST-EFFECTIVE THERAPY IN ONCOLOGY

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Aims: The integration of the pharmacogenetic data in the electronic clinical record and the development of a decision support system could be helpful for prescribing the most efficacious and cost-effective pharmacological therapy available. This is particularly relevant in oncology, where the therapeutic index of drugs is limited and the cost of therapies is high. The aim of the project is to radically innovate the prescriptive process of oncological medications by providing the prescriber an IT infrastructure for the automated management of the patient's molecular data that will be translated into specific indications.
Methods: The most up-dated literature data will be mined to extract a list of gene-drug interactions based on the most recent published guidelines to provide a list of the most relevant genetic information to be included in the patients clinical health record together with the corresponding pharmacogenetic guidelines.
Results: Public available guidelines were reviewed and compared to reach a consensus in terms of gene-drug pairs considered, their different level and classification of evidence rated basing on a scoring system, the therapeutic strategy recommended and the clinical impact of pre-emptive PGx test. Information on the strength of the recommendation (classification of recommendation, level of evidence and clinical impact) were considered necessary to be integrated in the prescribing system along with the therapeutic recommendation. A prototype of a decision support system is being developed through the partnership with two high tech companies active in Italy the healthcare system computerization.
Conclusion: Coordinated efforts through partnership are able to put together scientific and clinical expertise in the oncologic pharmacogenomic field, to implement the pre-emptive pharmacogenomic approach in the clinical practice in Italy, and to demonstrate its benefit in both patients clinical outcome and quality of life, with an economic advantage for the healthcare system.

NorPM-P20
MAJOR INFLUENCE OF THE CYP2D6*41 ALLELE ON CYP2D6 METABOLIZER PHENOTYPE – A STUDY ON 1,021 VENLAFAXINE-TREATED PATIENTS

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Aims: To compare the in vivo CYP2D6 metabolism encoded by the *9, *10 and *41 alleles, and assess the phenotype overlap between carriers of these variants with non-coding (null) alleles and null/null carriers (poor metabolizers, PMs).
Methods: The study included 1,021 patients from a therapeutic drug monitoring service performing both drug concentration analyses and genotyping. Absence of comedication with CYP2D6 inhibitors or antiepileptic enzyme inducers was confirmed for all patients. Metabolic ratio (MR) of O/N-desmethylvenlafaxine, a recently validated CYP2D6 biomarker, was matched with each patient's CYP2D6 genotype. In addition to *9, *10 and *41, the genotype assay comprised *3,*4,*5 and*6 null alleles, as well as gene duplication. MRs in carriers of *9, *10 or *41 combined with wild type (*1) or null alleles were compared by Mann-Whitney tests. In addition, the degree of subgroup overlaps in MR was calculated from direct observations, and a multiple linear regression analysis performed to provide MR subgroup estimates adjusting for covariates.
Results: The MR of O/N-desmethylvenlafaxine was lower in *41 than *9 or *10 carriers both combined with *1 and null alleles ($p < 0.002$). In *41/null carriers ($n = 30$), MRs of 26 patients (86.7%) were in the range of null/null carriers ($n = 95$) compared to three out of 17 carriers of *9/null or *10/null (17.4%). The multiple linear regression analysis provided MR estimates of 0.47, 1.33, 3.55, and 9.54, respectively, in carriers of null/null,*41/null, *9-*10/null, and*1/null ($n = 269$). In the multiple linear regression analysis, CYP2D6 genotype explained 60.7% of the overall variability in MR.
Conclusion: CYP2D6*41 encodes significantly lower CYP2D6 metabolism in vivo than *9 and *10. The majority of *41/null carriers express a CYP2D6 phenotype within the range of null/null carriers. Thus, pharmacogenetic assays for clinical routine and research should include CYP2D6*41 to capture most functional PMs.

NorPM-P21
PRELIMINARY RESULTS OF THE CLINICAL TRIAL GENOTYPE-PHENOTYPE CORRELATION OF DRUG-METABOLIZING ENZYMES

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Aims: To detect new genetic variants with impact on drug metabolism and to test the performance of genotyping and phenotype-prediction methods.
Methods: 200 healthy volunteers were exposed to a drug cocktail containing caffeine, losartan, omeprazole, dextromethorphan and midazolam. A blood sample was obtained after 5 h, urine was collected for 8 h. Subsequent measurement of the above mentioned drugs and their metabolites allowed calculation of metabolic ratios which were considered to show the activity of CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5 respectively. For genotyping, RFLP, qRT-PCR, pyrosequencing, Affymetrix DMET chips and Illumina exome SNP-chips were employed.

Results: Correlation of the Illumina chip data and the phenotype revealed 10 SNPs that were associated with changes in the metabolic ratio, most of them surprisingly not within the above-mentioned cytochrome p450 isoenzymes. Concordance of the different genotyping techniques was only in substantial to almost perfect agreement (κ Fleiss = 0.77–0.88, concordance 94.55% to 97.11%). In our study cohort, the DMET system could not predict ultra-rapid metabolizers of CYP2D6 and did not accurately predict poor-metabolizer phenotypes.
Conclusion: Genetic variants in other loci than P450s do not contribute significantly to the variability of metabolic ratios of the probe drugs. Due to broad variability however, a precise prediction of phenotypes using genetic data and by applying available algorithms is not readily possible for all P450 genotypes.

NorPM-P22
METHODOLOGICAL CHALLENGES FOR THE VALUATION OF DRUG RELATED TREATMENT COSTS FOR RELAPSING HAEMATOLOGIC CANCER PATIENTS

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Aims: For up to 3/4 of cancer patients, initial treatments are not effective, which leads into a trial and error treatment strategy resulting in unnecessary side effects and a waste of health care resources. However, little is documented about the costs associated with these secondary treatments while a large part of the limited hospital resources is used for them. Our goal is to develop health economics methods to calculate the cost in order to evaluate the potential savings of implementing precision medicine (PM).
Methods: Since January 1, 2016, relapsing haematologic cancer patients at Aalborg University Hospital have been offered to participate in the ProSeq protocol, where RNA- and DNA-sequencing are performed on the cancer cells to identify drug targets. Injectable (IJ) drugs dosages and prices were obtained from the hospital pharmacy combined with clinical data and dosages for non-IJ drugs from electronic health records. Data recordings end at death or lost to follow-up. The drugs were grouped by ATC codes and the price was estimated by a mg price based on the total number and price of packages and doses sold to the department. The total drug related treatment cost (DRTC) over the inclusion period was divided by the total days at risk to get an estimate of cost per day per patient.
Results: After 25 months, 208 patients have been included. Of these, 111 (myeloid leukemias = 11, lymphoid leukemias = 25, multiple myeloma = 16, lymphoma = 59) have received drug related treatment. The included patients have a median follow-up time (95% CI) of 11 months (9.6 months, 13 months) and one-yr survival rate of 76% (68%, 85%). Assuming 1 year stays in ProSeq the DRTC of relapsing patients was estimated to be 29 million DKK/year, which is 1/2 of the DRTC of all haematologic patients.
Conclusion: We have formulated a methodology to estimate DRTC. This will e.g. be used to 1) identify costly patients, 2) evaluate the benefit of implementing of PM and 3) aid in health care planning.

NorPM-P23
PHARMACOGENETIC MARKERS OF ADVERSE REACTIONS OF TYROSINE KINASE INHIBITORS – CASE REPORT

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Aims: Treatment of chronic myeloid leukemia (CML) is based on wide range of tyrosine kinase inhibitors (TKIs). The aim of this case-report is to describe patient with different tolerability to TKIs that could be explain by pharmacogenetic predisposition. According to literature, imatinib, nilotinib and dasatinib exhibit distinct interaction profiles with ABCB1 and ABCG2 transporters. For bosutinib literature is discordant whether it is substrate of ABCB1 transporter or not. Additionally, imatinib is metabolised by CYP2C9, CYP2D6, CYP3A4/5, while nilotinib, dasatinib and bosutinib by CYP3A4 enzymes.
Methods: A 69-year-old woman was prescribed dasatinib 80 mg/day. Patient developed following adverse drug reactions (ADRs) malaise, cough and dyspnoea and subsequently dose was reduced to 40 mg/day. Adverse drug reactions disappeared on lower doses and dose was increased to 60 mg/day and 80 mg/day, every second day but adverse reactions again reoccurred. Dasatinib was stooped and bosutinib was introduced in dose of 100 mg–400 mg/day without ADRs.
Results: Pharmacogenetic testing (PGX) revealed low expression of P-gp transporter and intermediate activity of transporter ABCC2 -24C/T and of enzymes CYP2D6 *1/*4 CYP2C9*1/*3 CYP2C19*1/*2 . Normal activity was observed for CYP3A4 (tested for*22) and ABCG2 (tested for 421C>A). Our results suggest that low activity of P-gp transporter could have contributed to the ADRs of dasatinib but did not cause ADRs of bosutinib.
Conclusion: This case suggests possible utility of pharmacogenetic testing to inform prescriber about the TKI with the lowest potential for ADRs for individual patient. Further research should be done to further elucidate pharmacogenetic predisposition for ADRs of certain TKIs.

NorPM-P24
OPPORTUNITIES AND CHALLENGES WITH DIRECT-TO-CONSUMER GENETIC TESTS IN GLOBAL AND NORWEGIAN PERSPECTIVE

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Aims: Direct-to-consumer genetic tests (GTs) have in recent years been gaining popularity globally and in the Nordic countries. The aim of this literature analysis was to investigate medical and legal challenges with GTs.
Methods: A search was performed in PubMed and Google Scholar from January 2010 to February 2018. Peer-reviewed original articles and reviews in English were included. EUR-Lex and Lovdata.no databases were used to identify relevant regulations in EU and Norway.
Results: GTs can be purchased at pharmacies or ordered by mail. The former are usually marketed as diagnostic for specific conditions like lactose intolerance, indicative towards individual's metabolic or athletic properties. None of these tests are currently sold by Norwegian pharmacies. GTs are also available for ordering online, and usually include information on predisposition to common diseases, carrier status for hereditary conditions, response to medications and genogeographic origin. The largest provider claims to have over 3 million customers. The major technological disadvantage of most GTs is that only panels of SNPs are sequenced, which reduces the costs but limits the coverage. Interpretation of GTs is complicated by the deficient scientific

evidence and lack of competence among medical professionals. There is little evidence that taking GTs gives health benefits. Transmission of personal genetic data to private companies is worrisome Among the EU countries legislation is diverse from virtually banning GTs to fully permitting them. In Norway the sale of GTs is not regulated by law, but testing of children is forbidden.
Conclusion: Most published studies deal with validity of GTs, bioethics, and legislation. Major challenges with GTs are deficient scientific evidence, low public and professional literacy in genomic health and vague regulations of GTs. GTs may contribute to early diagnostics and better health awareness, but progress is necessary in validating GTs and elaborating regulations.

NorPM-P25
VALPROATE AND PROTEIN BINDING – THE IMPORTANCE OF MEASURING FREE CONCENTRATIONS

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Aims: Routine use of therapeutic drug monitoring (TDM) of antiepileptic drugs by measurement of total concentrations is well established in epilepsy. In cases where changes in protein binding affect highly bound drugs (≈90% for valproate), measurement of the unbound concentration will be more accurate for exposure of active drug in the body. The aim of this study was to describe current practice where unbound serum concentrations of valproate were measured and to discuss the clinical impact in patients with epilepsy.
Methods: Retrospective, anonymous TDM-data from patients with measurements of unbound valproate concentrations were collected from the National Center for Epilepsy (2012–2017). The samples were taken drug-fasting in the morning at assumed steady-state. The study was approved by the Regional Ethics Committee.
Results: Data from 132 measurements in 81 patients using valproate were included, 40/60% women/men, average age was 39 (1–88) years. Hypoalbuminemia, reduced renal function, intensive care monitoring, adverse effects, displacement interaction with stiripentol, young/old age and pregnancy (5 pregnancies in 3 women) were relevant reasons for the request. Total serum concentration of valproate was 82–908 μmol/L (reference range 300–700), average unbound concentration 20% (5–93, reference ≈10%), and average dose was 1245 mg/day (72–3,000). Unbound concentrations of valproate increased up to 5-fold in two pregnancies and decreased post-partum, reflecting an increased exposure where use of total concentrations would be misleading.
Conclusion: Measurement of total and unbound concentrations of valproate showed increased free fraction in most of these vulnerable patients, and total concentrations may then underestimate drug exposure. Implementation of TDM with total and unbound concentrations may contribute to improved and tailored treatment for the individual patient.

NorPM-P26
GENOMIC AIDED PHARMACOTHERAPY (GAP): A PILOT STUDY IN PATIENTS SUFFERING FROM CHRONIC NON-CANCER PAIN

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Aims: Efficacy and safety of drugs vary significantly between individuals. The CYP450 enzyme system is responsible for the metabolism of most drug substances. During the recent years genetic testing has become more widely available. In Denmark the genetic test "Personalised Medicine Profile" (PMP) was recently made available by GeneTelligence as a tool to optimise and individualise pharmacotherapy. PMP is a genotyping test, which translates data on genetic variation and generates a personalised and evidence-based report on the influence of genetic variation on drug transport and metabolism by the CYP450 system. The report includes evidence based recommendations for individual therapy of 158 commonly prescribed drugs and identify the drugs that may cause significant side-effects or have reduced efficacy at standard doses. The patients' metabolic status is categorised in four classes: poor -, intermediate -, normal – and ultra rapid metabolisers.
The aim's of this pilot study was to evaluate the practical issues using the PMP test and the interpretation of the resulting report in a small selected population of patients suffering from chronic non-cancer pain and further to assess the number and types of therapy related challenges identified by the test.
Methods: Patients were recruited from two private Pain Centres in Denmark: Allévia and Kysthospitalet during January and February 2018. Eligible patients received a buccal swab kit. After performing the buccal swab, the kit was sent to GeneTelligence for DNA analysis. After genomic data translation the personalised evidence-based report is generated, using the PMP software platform. Based on this PMP report and the patients' current medication a pharmacist identified clinically significant drug therapy challenges and created a report containing recommendations for medication optimisation.
Results: Ten patients were included in this pilot study; the data analyses are still ongoing. The results will be presented at the 1.NORPM conference.
Conclusion: Conclusions will be presented at the meeting.

NorPM-P27
A CLINICALLY EFFECT OF UGT1A4*3 ON LAMOTRIGINE CONCENTRATION IS PREDOMINATELY OBSERVED IN POSTMENOPAUSAL FEMALES – A STUDY OF 534 PATIENTS

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Aims: Studies on therapeutic drug monitoring (TDM) of lamotrigine (LTG) have provided conflicting results regarding the impact of the UDP-glucuronosyltransferase (UGT) 1A4 genotype on the pharmacokinetics of LTG. As multiple factors may influence LTG pharmacokinetics, the present study aimed to investigate the impact of the *UGT1A4*3* variant on LTG serum concentration accounting for age, sex and valproic acid (VPA) comedication in a large patient population.
Methods: Matched TDM data on LTG and *UGT1A4* genotype of patients with information about VPA comedication were included retrospectively from a TDM service. Linear mixed model analysis allowing multiple measurements per patients was used to evaluate the impact of UGT1A4*3, as well as age, sex and VPA comedication, on dose-adjusted serum concentrations (C/D ratio) of LTG.
Results: Totally, 1735 LTG serum concentration from 534 patients were included. In the population, *UGT1A4*3* carriers had 10–15%

lower LTG C/D ratio compared to non-carriers ($p = 0.01$), but the quantitative impact of *UGT1A4*3* depended on age and sex. The difference was greatest in postmenopausal females (>50 years), where *UGT1A4*3* carriers obtained ~40% lower LTG C/D ratio than *UGT1A4*3* non-carriers ($p = 0.001$). The *UGT1A4*3* variant had only minor effect on C/D ratio in younger females (≤50 years) and males ($p > 0.1$). Regardless of genotype, patients with concomitant use of VPA had the most pronounced effect by increasing the LTG C/D ratio about 2.5-fold ($p < 0.001$).
Conclusion: This study shows that *UGT1A4*3* has a modest impact on LTG exposure, but it could lead to clinically relevant lowering in serum concentration among postmenopausal females. Thus, genotyping of UGT1A4 could be valuable for dose individualization when initiating LTG treatment in postmenopausal females.

NorPM-P29
P-GP INHIBITION INCREASES NEURONAL TOXICITY OF PACLITAXEL IN SH-SY5Y-DERIVED NEURONS

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Aims: Paclitaxel is an anticancer agent efficacious in the treatment of breast, ovarian and lung cancer. *ABCB1* polymorphisms have been associated with increased risk of paclitaxel-induced peripheral neuropathy. The aim of this study was to investigate the link between the drug efflux transporter P-glycoprotein (P-gp, encoded by *ABCB1*) and the neuropathy caused by paclitaxel.
Methods: Neuroblastoma SH-SY5Y cells were differentiated to neurons and treated with 0.1–10 μM paclitaxel for 24 h. Cytotoxicity of paclitaxel was assessed using the CellTiter-Glo® luminescence assay. The influence of P-gp inhibition during paclitaxel treatment was investigated by selectively inhibiting P-gp using 4 μM valspodar. Neurons treated with paclitaxel were immunolabeled with the neuronal marker tubulin beta III (TUBB3) and the number of neurites/cells were counted and neurite length measured using ImageJ.
Results: Paclitaxel caused a dose-dependent decrease in both number and length of neurites in SH-SY5Y-derived neurons, but did not cause cell death. In SH-SY5Y-derived neurons, 70% had more than two neurites after 0.1 μM paclitaxel exposure, while only 5% of the neurons had more than two neurites after 10 μM paclitaxel exposure. Neurite length was also significantly decreased in a dose-dependent manner ($p < 0.01$). Inhibiting P-gp led to a marked increased toxicity of paclitaxel when assessing both number and length of neurites ($p < 0.01$).
Conclusion: Paclitaxel caused a clear dose-dependent effect on number and length of neurites in SH-SY5Y-derived neurons that was exacerbated by inhibition of the efflux transporter P-gp. This may contribute to the understanding of the association of genetic variation in *ABCB1* and paclitaxel-induced peripheral neuropathy.

NorPM-P30
CLINICAL APPLICATIONS OF CYP450 GENOTYPING. SEVEN YEARS' EXPERIENCE IN A SWEDISH CLINICAL SETTING

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Aims: Genetics is a known source of interindividual variability in drug response. Polymorphic enzymes such as *CYP2D6*, *CYP2C19* or *CYP2C9* catalyse the oxidative metabolism of numerous commonly prescribed drugs, such as antipsychotics, antiepileptics, antidepressants and cardiovascular drugs. Evaluation of the metabolic capacity of an individual by genotyping may therefore help the clinicians to select the right dose and/or the best drug for a patient, thereby minimizing the risk of side-effects or therapeutic failure.
Methods: In the period 2011–2017 over 700 genotyping analyses were performed at our Department, with a constant yearly increase in the number of tests performed. The most requested test was *CYP2D6* (47%), followed by *CYP2C19* (33%) and *CYP2C9* (20%). Most patients were treated with psychoactive drugs (80%), such as antidepressants and classical antipsychotics, followed by cardiovascular, anticoagulant and antiplatelet drugs (15%). The principal reason for genotyping was lack of optimal drug response/therapeutic failure (60%), followed by side-effects (35%), while a few subjects were genotyped before starting therapy with drugs such as warfarin, clopidogrel, propafenone or tamoxifen.
Results: Ultrarapid metabolism (caused either by *CYP2D6* gene duplication or homozygosity for *CYP2C19*17*) was detected in a handful of subjects with therapeutic failure, suggesting that non-compliance plays a major role in therapeutic failure. Conversely, among subjects who had experienced side-effects, the frequency of detrimental alleles was higher than the background incidence. Furthermore, in some cases extensive metabolisers with side effects were phenotypically poor metabolisers, due to co-medication with potent CYP2D6 inhibitors such as paroxetine and bupropion.
Conclusion: Based on our experience, we conclude that genotyping is a valuable complement to plasma concentration determination when poor or ultrarapid drug metabolism is suspected.

NorPM-O1
EFFECT OF RARE SINGLE-NUCLEOTIDE VARIANTS ON THE BREAST CANCER RESISTANCE PROTEIN

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Aims: To study the effect of single-nucleotide variations in transmembrane regions and the first intracellular loop of the breast cancer resistance protein (BCRP, ABCG2).
Methods: Nine naturally occurring variants in the transmembrane region of BCRP (G406R, F431L, S441N, P480L, F489L, M515R, L525R, A528T and T542A) and five in the first intracellular loop (K453R, I456V, H457R, G462R and G462V) were selected. In addition, two common variants (V12M and Q141K) in the nucleotide binding domain were included as controls. The variant proteins were expressed using a baculovirus expression system in mammalian cells (HEK293). Crude membrane fractions of the cells were extracted to prepare membrane vesicles. Western blotting and immunofluorescence microscopy were used to study the expression and localization of the variants. The activity of the variants was tested using the vesicular transport assay with BCRP substrates Lucifer yellow and estrone sulfate. The data was used to predict in vivo effects of the BCRP variants on rosuvastatin pharmacokinetics in two PBPK models using Simcyp and MATLAB SimBiology.
Results: The transport activity of all variants in the transmembrane region was significantly impaired compared to wt BCRP, but two of the variants (K453R, I456V) in the intracellular loop retained comparable activity to wt BCRP. Decreased expression levels could explain the low activity for some, but not all, of the variants. Immunofluorescence studies in cells indicated impaired localization for some of the variants, in line with the activity and Western blot results. Based on the simulations, variants with impaired activity may increase the Cmax of rosuvastatin by two-fold and the AUC over three-fold compared to the wt BCRP.
Conclusion: Single nucleotide variations in the transmembrane region or the first intracellular loop may have destructive effects on BCRP activity, which may lead to unexpected pharmacokinetic events in patients carrying these variants.

NorPM-O2
DOXORUBICIN UPTAKE IN CARDIOMYOCYTES IS DEPENDENT ON ORGANIC CATION TRANSPORTER 3

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Aims: The anthracycline doxorubicin is among the most effective and commonly used anticancer agents, but its use is associated with a potentially life-threatening cardiotoxicity. We hypothesized that doxorubicin-induced cardiotoxicity (DIC) is initiated by transporter-mediated mechanism in which cells of the myocardium are selectively damaged, and that identification of the involved solute carriers can provide insights into the development of preventative strategies.
Methods: Transporter gene expression studies were conducted on human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) from patients that did or did not experience DIC. Determination of transport kinetics and mechanism of transport inhibition were performed in stably transfected HEK293 using TEA and metformin as prototypical substrates. Furthermore, doxorubicin disposition in *ex vivo* isolated cardiomyocytes and *in vivo* hearts were determined in wild-type and age-matched transporter-knockout mice.
Results: Multiple solute carrier genes were overexpressed by ≥2-fold in hiPSC-CMs from patients experiencing DIC, including OCTN1, OCT1, and OCT3. Among these transporters, deficiency of OCT3 in mouse cardiomyocytes was associated with the most significant decrease in doxorubicin uptake *ex vivo* ($p < 0.0001$). Accumulation of doxorubicin in cardiomyocytes of wild-type mice could be inhibited by known OCT3 inhibitors, including the tyrosine kinase inhibitors dasatinib and nilotinib, through a non-competitive mechanism. Consistently, heart levels were reduced in OCT3-knockout mice after doxorubicin administration *in vivo*.
Conclusion: We identified a previously unrecognized pathway of DIC that is initiated by an organic cation transporter system and highly sensitive to pharmacological inhibition. These findings shed further light on the etiology of DIC, and provide a rationale for the future development of new targeted interventions to mitigate this debilitating side effect.

NorPM-O3
MICRORNA-155 CONTROLS VINCRISTINE SENSITIVITY AND PREDICTS SUPERIOR CLINICAL OUTCOME IN DIFFUSE LARGE B-CELL LYMPHOMA

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Aims: A major clinical challenge of diffuse large B-cell lymphoma (DLBCL) is that 30–40% of patients have refractory disease or relapse after initial response to therapy, due to drug-specific molecular resistance. The purpose of this study was to investigate miRNA involvement in vincristine resistance in DLBCL.
Methods: By analyses of miRNA microarrays from DLBCL cell lines with different vincristine sensitivity, we identified lower miR-155 expression in resistant cells. This association was further examined by functional *in vitro* analysis, studying the direct impact of miR-155 in vincristine response, and by target gene analysis. Secondly, prognostic impact of miR-155 expression was evaluated in two independent DLBCL cohorts.
Results: Exogenous upregulation of miR-155 sensitized GCB-DLBCL cells to vincristine, and consistently, downregulation and knock-out of miR-155 induced vincristine resistance, documenting that miR-155 functionally controls vincristine sensitivity. Target gene analysis identified Wee1, a cell-cycle checkpoint gene, as a target of miR-155 in DLBCL. Chemical inhibition of Wee1 sensitized GCB-DLBCL cells to vincristine, suggesting that miR-155 controls vincristine response through Wee1. Clinical outcome analysis of DLBCL patients revealed that high miR-155 expression level was significantly associated with superior survival for R-CHOP treated patients of the GCB subclass, independent of the well-established international prognostic index (IPI), an observation challenging the commonly accepted perception of miR-155 as an oncomiR.
Conclusion: We experimentally confirmed a direct link between high miR-155 expression and vincristine sensitivity in DLBCL and documented an improved clinical outcome of GCB classified patients with high miR-155 expression level. This suggests that the role of miR-155 in vincristine response is important enough to affect overall survival.

NorPM-O4
THE PROTEIN KINASE INHIBITORS MIDOSTAURIN AND NINTEDANIB ARE TIME-DEPENDENT INHIBITORS OF CYP3A4

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Aims: Protein kinase inhibitors display a tendency to affect cytochrome P450 (CYP) 3A4 by time-dependent inhibition. As CYP2C8 and CYP3A4 share overlapping substrate specificity, we tested six novel kinase inhibitors for time-dependent inhibition of these enzymes.
Methods: The inhibitory effects of dovitinib, masitinib, midostaurin, nintedanib, trametinib and vatalanib on amodiaquine N-deethylation (CYP2C8) and midazolam 1'-hydroxylation (CYP3A4) were evaluated in human liver microsomes. Static predictions were used to estimate the clinical significance of the observed inhibition.
Results: Dovitinib, midostaurin and nintedanib exhibited time-dependent inhibition of CYP3A4 (IC_{50} shift >1.5), whereas masitinib, trametinib and vatalanib did not affect CYP2C8 or CYP3A4 by time-dependent inhibition (IC_{50} shift <1.5). Further experiments identified midostaurin and nintedanib as mechanism-based inhibitors of CYP3A4, with maximal inactivation rate (k_{inact}) and inhibitor concentration supporting half of k_{inact} (K_I) values of 0.052 1/min and 2.72 μM, and 0.025 1/min and 17.3 μM, respectively. Predictions indicated that standard doses of nintedanib are unlikely to cause drug interactions with CYP3A4-dependent substrates, whereas midostaurin could increase the plasma exposure to such substrates several-fold. Furthermore, based on reversible inhibition, masitinib and vatalanib were predicted to increase the plasma exposure to sensitive CYP2C8 and CYP3A4 substrates by ≥2-fold.
Conclusion: Our data identifies two additional kinase inhibitors as time-dependent inhibitors of CYP3A4, and detects a risk for drug interactions between several of the tested inhibitors and CYP2C8 and CYP3A4 substrates.

NorPM-O5
GENOME-WIDE ASSOCIATION STUDY OF PANDEMRIX-INDUCED NARCOLEPSY IN SWEDEN – A POSSIBLE ROLE FOR GLIAL DERIVED NEUROTROPHIC FACTOR (GDNF)

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Aims: Narcolepsy is an autoimmune disease characterized by an inability to control sleep and wakefulness. The number of young

diagnosed with narcolepsy rose sharply following immunization with the A(H1N1)pdm09 influenza virus strain vaccine Pandemrix in Sweden 2009–2010. The most frequent form, narcolepsy type I, is known to be caused by a loss of hypocretin neurons, and is strongly associated with *HLA-DQB1*0602*. However, only 0.02 % of *HLA-DQB1*0602* carriers developed narcolepsy following vaccination. We sought to determine whether other genetic factors contribute to the risk of narcolepsy associated with Pandemrix.

Methods: 43 adjudicated cases of Pandemrix-associated narcolepsy from the Swedish adverse drug reaction biobank Swedegene were compared with 4891 Swedish population controls from TwinGene. Genotyping was performed on Illumina arrays. The merged dataset contained 600 K single nucleotide polymorphisms (SNPs), and after phasing and imputation 8.6 million SNPs. We corrected for principal components 1–4. The genome-wide significance *p*-value threshold was set to $p < 5 \times 10^{-8}$.

Results: Narcolepsy was significantly associated with *HLA-DQB1*0602*, odds ratio (OR) 6.4 [95% confidence interval (CI) 4.2, 9.8], $p = 1.4 \times 10^{-17}$. After correction for *HLA-DQB1*0602*, the strongest association was with the glial cell line-derived neurotrophic factor antisense gene (*GDNF-AS1*), OR=8.6 [95% CI 4.2, 17.6], $p = 2.6 \times 10^{-9}$.

Conclusion: We found an association with an antisense RNA that is transcribed from the opposite stand of the glial cell line-derived neurotrophic factor gene (*GDNF*). The antisense transcript has been shown to regulate the expression of *GDNF*, which encodes an essential neurotrophic factor that supports neuronal survival. Dysregulation of *GDNF* has been seen in brain cells from patients with Alzheimer's and Parkinson's disease. This finding may increase the understanding of mechanisms behind vulnerability to narcolepsy.

NorPM-O6
CLINICAL IMPLEMENTATION OF PERSONALIZED MEDICINE BY NOVEL APPROACHES IN THERAPEUTIC DRUG MONITORING (TDM)

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Aims: One of the most important aspects of personalized medicine is the identification of optimal dose. While inter-patient variability in pharmacokinetics is substantial and multifactorial, high-precision predictive markers of the optimal individual dose are often lacking. Bioanalysis of drug/drug metabolite concentrations will play an important role for individual dosing and the aim of this presentation is to summarize recent developments in TDM methodology.

Methods: There has been a dramatic development in bioanalysis based on mass spectrometry (MS). Multi-component detection methods with high accuracy and precision are now clinical routine. High-resolution (HR-)MS will allow for detection and quantification of essentially all low-molecular weight molecules in a single sample. Obvious applications include intoxications, monitoring of polytherapy and patient compliance. New sampling methodology include dried blood spots and exhaled breath particles derived from lung epithelial lining fluid. Population pharmacokinetics-based models will improve area-under-the-concentration-time-curve (AUC) estimations and increase flexibility in sampling time.

Results: There is an increasing demand of the Karolinska TDM laboratory with last year approaching 75,000 analyses including around 1,300 genotyping requests on TPMT or CYP. At present, we quantify the systemic concentration of 120–150 different agents. The major therapeutic areas are immune-modulating drugs, antiepileptics, antimicrobials and psychoactive drugs. Much work is also spent on IT-solutions for correct in-data and reports. TDM-sampling indications are specified together with clinicians, and results interpretations rely on updated reviews of the scientific literature.

Conclusion: TDM is under strong development and receives an increased clinical interest. TDM provides the tool for more precise dose adjustments in the individual case, and may work well in tandem with pharmacogenetic approaches that aim to individualize starting dose.

NorPM-O7
SELECTING PHARMACOGENETIC TEST FOR CLOPIDOGREL PATIENTS

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Aims: Clopidogrel is a pro-drug which is metabolized by CYP2C19 to an active substance. About 30% of European population are poor or intermediate CYP2C19 metabolizers. For them the effect of clopidogrel may be absent or poor and testing of CYP2C19 is therefore recommended. Patients using clopidogrel often use other drugs as well. Based on epidemiological data we evaluated, how often the patient would benefit from a larger pharmacogenetic profile compared to CYP2C19 definition alone.

Methods: From Finnish prescription statistics we collected all prescriptions from over 30 cities in Finland. It covered 20% of the Finnish population. All patients who had received clopidogrel prescription in 2015–2017 were selected. From these patients we calculated, how many patients had received in the same period a drug where defining CYP2D6 or SLCO1B1 is recommended. According to Finnish database the substances where CYP2D6 is recommended are: amitriptyline, codeine, metoprolol, nortriptyline, paroxetine, risperidone and tamoxifen. SLCO1B1 is recommended for simvastatin patients.

Results: In the material we found 17.373 patient who had a clopidogrel prescription. 6,547 patients (37.7%) had only clopidogrel prescriptions. 5,105 patients (29.4 %) had at least one prescription of drugs where CYP2D6 is recommended. The four most commonly used CYP2D6 drugs in the clopidogrel population were: metoprolol (2,089), codeine (1,876), amitriptyline (1,037) and risperidone (674). In the population we had 5,503 patients (31.7%) who had received simvastatin.

Conclusion: Clopidogrel patients often have other drugs where pharmacogenetics testing would be beneficial. Based on our data it is evident, that the population would benefit from larger set of pharmacogenetic tests. At least CYP2D6 and SLCO1B1 are relevant in this patient population. When the test is performed from the same sample, the additional testing cost is minimal compared to the benefit for the patient and health care payers.

NorPM-O8
GLP-1 RECEPTOR VARIANTS MARKEDLY DIFFERENTIATE GLYCAEMIC RESPONSE TO GLP-1 RECEPTOR AGONISTS: A DIRECT STUDY

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Aims: Glycaemic response to GLP-1 Receptor Agonist (GLP-1RA) treatment varies markedly among patients with Type 2 Diabetes (T2D)

yet the mechanism for this variation is uncertain and only short-term glycaemic response can predict who will have a merked mid-long-term response and who will respond less. Common missense variants in the GLP-1R have previously been reported to alter GLP-1 mediated insulin secretion in non-diabetic individuals. We aimed to investigate how GLP-1R variants alter glycaemic response to the GLP-1RA.

Methods: We performed a meta-analysis across the DIRECT, PRIBA and GoDARTS cohorts. A total of 1,156 T2D subjects were followed-up for 6 months after initiation of GLP-1RA treatment. The association of GLP-1R variants, rs6923761 (Gly168Ser) and rs10305420 (Pro7-Leu), with reduction in glycated haemoglobin (HbA1c) after treatment were assessed using multiple linear regression in an additive model.

Results: Gly168Ser and Pro7Leu variants were independently associated with reduced efficacy of GLP-1RA to lower HbA1c (Gly168Ser β (HbA1c change per allele) = −0.18%, $p = 0.001$, Pro7Leu β = −0.14%, $p = 0.01$). We then derived a genetic risk score, summing up these two variants. The 22% of the population who carry no variant allele in either of the variants had a mean HbA1c reduction of (1.39%[1.21–1.58] (15.22 mmol/mol [13.17–17.27])). In contrast the 17% percent of the population who carry 3 or more risk alleles had a much lower glycaemic response (0.86% [0.66–1.13] (9.4 mmol/mol [7.00–11.92])), a difference of 0.53% [5.8 mmol/mol] ($p < 0.001$). There was no significant impact of these SNPs on weight change in response to GLP-1RA suggesting the variants impact on the glycaemic lowering mechanisms of GLP-1RA rather than weight lowering mechanisms.

Conclusion: Missense variants in the GLP-1R have a large clinical impact on glycaemic response to GLP-1RA. The frequencies of these variants are not rare, making this the largest, most common pharmacogenetic effect described to date in T2D.